

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 1416, 1610, 1100		(11) International Publication Number: WO 00/61618	
A1		(43) International Publication Date: 19 October 2000 (19.10.00)	
(21) International Application Number: PCT/CA00/00338	(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, GR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AR, JP, patent (G), GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), European patent (A), AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TO).		
(22) International Filing Date: 5 April 2000 (05.04.00)	(30) Priority Date: 09/28/942 13 April 1999 (13.04.99) US	Published With international search report.	
(71) Applicants (for all designated States except US): UNIVERSITY OF TORONTO (CA/CA), Medical Sciences Building, 1 Kings College Circle, Toronto, Ontario M5S 1A8 (CA), CONNAUGHT LABORATORIES LIMITED (CA/CA), 1755 Steeles Avenue West, Toronto, Ontario M2K 3T4 (CA).			
(72) Inventors and (73) Inventors/A applicants (for US only): P.A. Emil, F. DE/CA), 51 Dundas Drive, Toronto, Ontario M8X 2K7 (CA), KLEIN, Michael, H. (CA/CA), 16 Memo Boulevard, Willowdale, Ontario, M2P 1B9 (CA), CHONG, Pele (CA/CA), 32 Esplanade, Richmond Hill, Ontario L4C 0B6 (CA), PEDY-CZAK, Arthur (CA/CA), 1399 Colmar Avenue, Pickering, Ontario L1W 1C2 (CA).			
(74) Agent: STEWART, Michael, I.; Sim & McBurney, 6th floor, 330 University Avenue, Toronto, Ontario M5G 1R7 (CA).			
(54) Title: Fab-EPTOPE COMPLEX FROM THE HIV-1 CROSS-NEUTRALIZING MONOCLONAL ANTIBODY 2F5			
(57) Abstract <p>The crystal structure of the Fab' fragment of H4b 2F5, a potent neutralizer of both laboratory strains and primary clinical isolates of HIV-1, both uncomplexed and complexed with the highly conserved peptide sequence ELDKVAS of the viral envelope protein gp41, has been elucidated and the characteristics of peptide-protein interactions determined. Having regard to such determination, the peptide-antimetals are constrained in the three-dimensional structure to provide an increased immunogenicity to the epitope sequence.</p>			

W/O 00/61618

PCT/CA00/00338

2

The elucidation of these three-dimensional structures enables there to be constructed, as set forth herein, peptide-mimetics constrained in the same β -turn-like configuration as seen in the crystal structure of the complex, which would be expected to increase the immunogenicity of the epitope sequence.

Accordingly, in one aspect of the invention, there is provided an isolated crystal of the Fab' fragment of monoclonal antibody 2F5. The isolation of the crystalline form of the Fab'2F5 fragment enables the three-dimensional structure of such form of the fragment to be determined and such structure is shown in Figure 1, described below. Certain characterizing parameters have been determined for the crystal structure, as set forth in Table 2 below.

The isolated crystal may be grown in space group P2₁2₁2, with cell dimensions $a=63.6$ Å, $b=76.4$ Å, $c=93.4$ Å, although the crystals may be grown in another space group with its own unique cell dimensions. The crystalline form of the Fab'2F5 may have the atomic coordinates deposited on April 9, 1999 with the Protein Data Bank under Accession No. 2F5A.

Fab'2F5 molecules organized in the isolated crystal provided herein possess a third hypervariable (V3) loop of the heavy chain comprising amino acid residues H98 to H120, as seen in Table 1 below, which has a three-dimensional structure as shown in Figure 4, described below and atomic coordinates as shown in Table 3 below.

In accordance with a further aspect of the present invention, there is provided an isolated crystal of the Fab' fragment of monoclonal antibody 2F5 complexed with a peptide having the amino acid sequence ELDKVAS (SEQ ID No. 1) or a functional analog thereof. The solution of the crystal form of the complex enables the three-dimensional structure of such form of the complex to be determined and the detail of the binding site of the peptide to the Fab' fragment is shown in Figure 3, described below. Certain characterizing parameters have been determined for the crystal structure of the complex, as set forth in Table 2 below.

The isolated crystal complex may be grown in space group P2₁2₁2, with cell dimensions $a=58.0$ Å, $b=65.0$ Å, $c=175.6$ Å, although the crystal complex may be grown in another space group with its own unique cell dimensions. The

3

crystalline form of the complexed form of the Fab2F5 may have the atomic coordinates deposited with the Protein Data Bank under Accession No. 2F5B on April 9, 1999.

The functional analog of the amino acid sequence ELDKWAS may be one in which lysine is replaced by arginine and/or one in which tryptophan is replaced by tyrosine, phenylalanine or uncharged histidine. One example of such functional analog is ELDRWAS (SEQ ID No: 2).

The elucidation of the crystal structure of the Fab2F5 fragment when bound to the peptide ELDKWAS (SEQ ID No: 1), provides details of the actual conformation of the peptide epitope when it is bound to the antibody, which will be the same, irrespective of the kind of crystal which is analyzed.

The information which is provided concerning the conformation of peptide epitope then provides the basis for the provision of peptide analogs, peptide mimetics and other antigens which are potentially useful as components of an anti-

15 HIV vaccine.

Accordingly, in another aspect of the present invention, there is provided a synthetic peptide which binds to monoclonal antibody 2F5 and which is constrained to provide a three-dimensional structure corresponding to that for the peptide ELDKWAS (SEQ ID No: 1) shown in Figure 3.

20 This synthetic peptide may contain the amino acid sequence DKW or a functional analog thereof and may be constrained in the slightly distorted β -turn configuration of the three-dimensional structures with the tryptophan and lysine residue chains stacked and parallel, as seen in Figure 3 and as discussed in more detail below.

25 The analysis of the three-dimensioned conformation of the epitope indicates that at least one of the tryptophan and lysine sidechains may be substituted by an amino acid which retains the peptide-protein interaction shown in Figure 3, which also binds to the Mab. For example, arginine (R) may be used in place of lysine (K) and tyrosine (Y), phenylalanine (F) and uncharged histidine (H) may be used in place of tryptophan (W). Peptides wherein one or more of such amino acid substitution is effected are peptides which contain a "functional

4

analog" of the amino acid sequence DKW, as the term is understood herein, in that the peptide still binds to the monoclonal antibody 2F5.

The synthetic peptide provided herein may be constrained in the required conformation by any convenient means. For example, a disulphide bridge may be used to maintain the amino acid sequence DKW or analogs thereof in the respective orientation of two amino acid residues as shown in Figure 3. Such disulphide bridge may be provided between cysteine residues in the synthetic peptide ECDKWCS (SEQ ID No.: 3).

Alternatively, a lactam bond may be used to maintain the amino acid sequence DKW or functional analogs thereof in the respective orientation of the amino acid residues as shown in Figure 3. Such lactam bond may be formed between diaminopropionic acid (Dap) and glutamate (E) residues in the synthetic peptide EdapDKWES (SEQ ID No.: 4) or EEDKWWDaps (SEQ ID No.: 5).

15 It is well known that the immunogenicity of peptides may be enhanced by conjugation to carrier molecules, such as proteins, including diphtheria toxoid, tetanus toxoid or an outer membrane protein of *Haemophilus*. Such carrier protein may be linked to the peptide.

There is also provided, in an additional aspect of the invention, a method of making a peptide binding to monoclonal antibody 2F5, which comprises co-crystallizing a Fab' fragment of the monoclonal antibody 2F5 with a peptide having the amino acid sequence ELDKWAS (SEQ ID No.: 1) or functional analog thereof to form a crystalline complex; analyzing the crystalline complex to determine the three-dimensional orientation of the bound peptide in relation to the Fab' fragment; and synthesizing a peptide containing at least amino acids DKW or functional analogs thereof constrained in the determined three-dimensioned orientation.

25 The functional analog of the peptide containing at least amino acids DKW is one which still binds to the monoclonal antibody 2F5. Functional analogs also extend to known analogs of the ELDKWAS motif, including those of the formula X_1LDKW_X2S wherein X_1 is E, A, G or Q and X_2 is A or T.

BRIEF DESCRIPTION OF THE DRAWINGS

The file of this patent application contains drawings executed in color, namely Figures 1 to 4. Copies of this patent with color drawing(s) will be provided by the Patent and Trademark Office upon request and payment of the necessary fee.

Figure 1 is a colored ribbon diagram of crystalline Fab 2F5, showing the heavy chain in purple, the light chain in blue and the elongated VH3 loop (colored in gold) extending from the protein surface, as generated by MOLSCRIPT (ref. 27) and Raster 3D (ref. 28);

Figure 2 is a colored stereoplot of the ELDKWAS peptide model in density, as generated by the program O (ref. 29). The Fo-Fc map was calculated with the peptide omitted and contoured at 3 σ . A minor break in the density at P7-Ser at the contour level illustrates the slight increase in flexibility at the extremes of the bound epitope;

Figure 3 is a color representation of the antigen binding site of Fab 2F5, showing protein/peptide interactions, as generated using the program SETOR (ref. 30). The residues are colored by atom type: oxygen is red, nitrogen is blue, carbon is grey and sulfur is yellow. For clarity, some hydrophobic sidechains which interact with the epitope have been omitted. All bond lengths are given in Å, and

Figure 4 is a color representation of the third hypervariable loop of the heavy chain of Fab 2F5 complex comprising amino acid residues H98 to H120, as generated using the program SETOR (ref. 30). The residues are colored by atom type.

GENERAL DESCRIPTION OF INVENTION

The crystalline structure of the Fab' fragment of Mab 2F5 (IgG) was solved at 2.05 Å resolution by molecular replacement and adopts the standard immunoglobulin fold. A salient feature of the structure is the elongated (22 amino acids) hypervariable loop 3 of the heavy chain (V-H3, ref. 9), which comprises amino acid residues H98 to 120 and extends away from the protein surface, as can be seen from the ribbon diagram of Figure 1. The V-H3 loop is shown in detail in Figure 4. The atomic coordinates of the V-H3 loop are given in Table 3.

In the structure of the Fab 2F5 complex with bound epitope, refined at 2.0 Å, this loop is well-defined by clear electron density. In the uncomplexed form, while the V-H3 region is less clear, loops at the C-terminal regions of the heavy chain constant domain, including the C-termini of both chains, were better resolved. Conformations from the better-defined electron density were used as templates for building the other model. The refined models comprise residues L1 to L214 of the light chain and residues H1 to H146 and H151 to H235 of the heavy chain plus ordered water molecules. The amino acid sequences of the light chain (SEQ ID No.: 2) and heavy chain (SEQ ID No.: 3) of Fab 2F5 are shown in Table 1 below. For the structure of the complex, P1 to P7 are the residues of the peptide. The H147 to H150 loop of the constant domain of the heavy chain was not visible in either structure. (Residues are labelled herein H1 to H235 for the heavy chain and L1 to L214 for the light chain and P1 to P7 for the peptides).

Along with differences in mobility of the loops mentioned above, the elbow angle in the complexed form differs from uncomplexed Fab 2F5 (142° vs. 146°). Both of these observations may be artifacts of crystal packing, since the unit cells are different, uncomplexed Fab 2F5 having a unit cell which is 30% smaller. An overlay of all C α atoms results in an rmsd of 0.7 Å, but these shifts appear to be the result of a concerted domain movement (i.e. the change in elbow angle) rather than any modification of the antigen binding site. Superpositioning only the variable regions gives an rmsd of 0.4 Å. While the results of the structural analysis do not provide any obvious explanation for the long insertion in the V-H3 loop has been identified, its unusually hydrophobic nature for surface residues suggests it plays a role in the antibody mechanism. It may be involved in interactions with a portion of gp41 C-terminal to the epitope sequence, enhancing binding and increasing the specificity of the Fab. It may even form an integral part of the neutralization mechanism, perhaps by disrupting the conformation of the gp41 coiled-coil trimer.

In the complexed structure, the ELDKWAS peptide forms a slightly distorted, type I β turn, centered between P4-Lys and P5-Trp, (as seen in Figures 2 and 3), with a 3.1 Å hydrogen bond from the amide nitrogen of P6-Ala to the carbonyl oxygen of P3-Asp. The arrangement is atypical in that neither position

7

two or three in the turn is a glycine (ref. 10), but rather the bulky residues lysine and tryptophan. The dihedral angles for P5-Trip fall in the "unfavoured" region of a Ramachandran plot ($\phi=101.7^\circ$, $\psi=8.7^\circ$).

Another interesting feature of the complexed structure is the stacked arrangement of the adjacent P5-Trip and P4-Lys sidechains, with hydrophobic interactions between the fully-extended alkyl chain of the P4-Lys and the aromatic rings of P5-Trip at a distance of about 3.8 Å. The lysine sidechain, whose hydrophobic methylene groups are sandwiched between P5-Trip and H54-Tyr, ends with a sharp turn at the final amino group, forming contacts with H56-Asp and H58-Asp. While the principal hydrophobic contacts of P5-Trip are the P4-Lys methylene groups, other hydrophobic residues within 4 Å of the aromatic ring system include H103-Pro and H32-Phe and the methylene groups of the sidechain of H113-Arg. A key component to the stability of the peptide configuration is the orientation of the P3-Asp sidechain, which forms strong hydrogen bonds to the backbone amide of P5-Trip as well as to L96-His-Nε and H100-Arg-NH1, all about 2.8 Å long. A water molecule associated with P5-Trip-Nε1 at 3.0 Å also forms strong hydrogen bonds to backbone carbonyls of H33-Gly and H101-Arg at 2.7 and 2.8 Å respectively. From this analysis, it can be concluded that the Asp-Lys-Trip (DKW) trio are the essential component of the protein/peptide interaction.

This conclusion is supported by mutation studies in which changes outside the DKW core do not have a dramatic effect on binding, whereas major modifications within the trio usually prevent neutralization (ref. 5). It was estimated that the LDKW motif is 83% conserved among HIV-1 envelope glycoprotein sequence (ref. 4). For the critical portion of the epitope, DKW, conservation among 206 sequenced HIV-1 envelope proteins of all clades in the 1997 to 1998 Los Alamos HIV Sequence Database (ref. 11) is 86%. Within the B clade, conservation is 92% (91/99 sequences). Phage library screening with Mab 2F5 also selected sequences with a DRW core (ref. 4). The structure of a complex where an arginine is substituted for P4-Lys (i.e. peptide ELDRWAS (SEQ ID No: 2)) shows identical peptide conformation and contacts as the complex reported here with the consensus epitope. The total buried accessible surface area upon

8

formation of the complex is 1025 Å² (calculated as the difference in accessible surface between the intact complex and the sum of the surface areas of the peptide and uncomplexed Fab' determined using a probe of radius 1.4 Å (ref. 12)). The peptide coordinates of the complex Fab'2F5 + ELDKWAS are shown in Table 4 while those for the complex Fab'2F5 + ELDRWAS are shown in Table 5.

The conformation of the gp41 epitope found in the complex with Fab'2F5 and seen in detail in Figure 3 was not anticipated. A helical conformation had been proposed (ref. 13) which was consistent with an extension of the observed coiled coil of the gp41 ectodomain (refs. 14 to 19). Most structural analyses of HIV-1 (refs. 14 to 16) or SIV (refs. 17 to 19) gp41 do not incorporate the epitope sequence, although two reports (refs. 14, 19) include a partial sequence. In one (ref. 14), ELD at the C-terminus of the crystallized portion adopted an α -helical structure, the continuation of a long (37 aa) helix. In the other, the C-terminus is an unstructured coil (ref. 19).

A conformation of the full epitope was determined as part of a fusion protein, where it was connected to the C-terminus of glutathione-S-transferase (GST) by a nine amino acid linker (ref. 20). In this environment, the epitope formed part of a series of tight turns but not the β -turn seen in the results described herein. In the GST-fusion structure, the epitope peptide interacted with a neighboring molecule in the crystal, making it probable that crystal packing forces led to the observed conformation. The gp41 peptide portion of the structure also had high thermal parameters, denoting flexibility.

Preliminary NMR studies have suggested that the ELDKWAS sequence adopts very little or no stable secondary structure. The crystal structure of Fab'2F5 elucidated herein explains the stronger immune response observed when the epitope was introduced into loops of hemagglutinin (refs. 2, 21) or recombinant antibodies (ref. 22) where a β -turn conformation might be induced, in contrast to hepatitis B virus surface antigen (ref. 8), where epitope grafting resulted in an excellent humoral response of 2F5-like binding specificity but failed to neutralize live virus, underlining the importance of the correct epitope conformation.

The conformation of the gp41 epitope set forth herein may be adopted transiently, after assembly of the mature gp41/gp120 trimers on the virus

envelope, or possibly during the fusion process. A range of conformations for gp41, including the stable fusogenic form observed in the structural determinations made herein, as well as an intermediate "unspun" and non-fusogenic form has been proposed by several investigators (refs. 14, 23). A short life span of the antigen would be consistent with its low immunogenicity and the consequent absence of Mab 2F5 in the antisera of most infected patients. As well, passive immunization with Mab 2F5 in chimpanzees failed to neutralize HIV-1, resulting in delayed infection and lower viral loads, but not protection (ref. 6). This result was presumably due to insufficient opportunity for antibody binding, either because of low antibody concentration or the short lifetime of the antigenic conformation. As the only identified cross-neutralizing antibody against gp41, Mab 2F5 is an important focus in HIV-1 vaccine research. It is one of only three broadly neutralizing monoclonal antibodies identified to date and the only one with a short, continuous epitope. The other two known cross-neutralizing Mab's are b12 and 2G12 which define epitopes at the CD4 binding site and V3/V4 loops of gp120 respectively (ref. 6), but in these cases the epitopes are discontinuous and involve both peptide and carbohydrate interactions (refs. 5, 6).

Development of a peptide-mimetic constrained to adopt the conformation of the gp41 sequence found in the structure of Fab 2F5 could overcome the low immunogenicity of the epitope, making such a compound a useful component of a future HIV-1 vaccine.

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, peptide-mimetics chemistry, protein biochemistry, crystallography and immunology used but not explicitly described in

this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

Example 1

This Example shows the preparation, purification and crystallization of Fab 2F5 and its epitope complex.

Intact human 1A8 2F5 IgG antibody was transformed into F(ab')₂ using standard pepsin protocols. F(ab')₂ was then stored with 1% (w/v) clinical human albumin added to the solution for stability. To separate the protein from the albumin, DE52 cellulose was swollen in 20mM Tris pH 8.0 and packed into a column 3 cm wide, 5 cm high, providing about 30 mL bed volume. The column was washed overnight with 2 L of 20 mM Tris pH 8.0.

55 mL protein at 1.1 mg/mL concentration were dialysed against 2 x 4 to 5 L of 20 mM Tris pH 8.0 and the conductivity and pH of the buffer, flow through and protein concentration were checked to ensure the protein bound to the column. The protein was loaded onto the column by pumping on at 1 to 5 mL/min, with albumen binding to the column while the F(ab')₂ does not. Buffer A (20 mM Tris pH 8.0) was run through the column until the OD₂₈₀ went down to baseline and approximately 7 mL fractions were collected.

The albumin was eluted with a salt gradient of 20 mM Tris pH 8.0, 20 mM Tris pH 8.0 + 0.2 M NaCl, to ensure no other proteins were present. The flow-through protein was concentrated, producing 5 x 500 μ L of F(ab')₂ at 23 mg/mL. The sample was confirmed to be F(ab')₂ by reducing and non-reducing native and SDS-PAGE gels as well as by a positive antigen-catch ELISA assay targeting the k-chain followed by a negative assay targeting the Fe part of a human antibody molecule.

200 μ L of Fab' at 23 mg/mL were diluted to 4 mL with 0.1 M Tris pH 8.0, 400 μ L 100 mM DTT in 0.1 M Tris pH 8.0 were added to the 4 mL to provide a final concentration of 10 mM in DTT. The solution was incubated at room temperature for an hour, 30 μ L of a 500 mM iodoacetamide solution in 0.1 M Tris pH 8.0 were added and the solution left for a further 30 minutes. The Fab' was dialyzed overnight against 20 mM Tris pH 8.0 and concentrated to 10 mg/mL for use in crystallization setups.

11

Crystals of uncomplexed Fab' grew from hanging drops of 5 mg/ml protein with 1.0 M ammonium sulfate at pH 5.8 as precipitant and grew as needles. Complexes were co-crystallized by adding a 3:1 ratio of peptide ELDKWAS to protein and incubating overnight before setting up as hanging drops of 5 mg/ml complex at pH 5.8, using 1.6 M ammonium sulfate at pH 7.0 as precipitant. The crystals grew in two days as large square bipyramids.

The sequence of the heavy and light variable domains has recently been published (ref. 10) and agrees with the one used in this study with a single correction at amino acid H110, which is a serine rather than a proline as originally stated. The full amino acid sequences of the variable and constant domains of the Fab' fragment are shown in Table 1 below (SEQ ID Nos: 6 and 7).

Crystals of the free Fab' belong to the space group P2₁-2₁ (unit cell: a=63.6 Å, b=76.4 Å, c=94.7 Å) and grow as needles. Crystals of the complex also adopt space group P2₁-2₁ (unit cell: a=59.0 Å, b=65.0 Å, c=175.6 Å) and grow as square bipyramids. Crystals were flash frozen for data collection. Data were collected on a Rigaku FR-C equipped with Molecular Structure Corp mirror optics and with a Mar345 image plate detector (Fab'2F5) and at the National Synchrotron Light Source in Brookhaven using a Mar30 detector (complex). Data were processed using DENZO and SCALEPACK (HKL Research).

20 Example 2

This Example describes the solution of the structure of the Fab'2F5 complexed and uncomplexed.

The structure of the Fab'2F5 complex was solved by molecular replacement (ref. 24) using PDB entry 1CLZ (ref. 25) minus sidechains and hypervariable loops as the search model. Constant and variable regions were used as independent models. The correct solution had a correlation coefficient of 35.3 (R=47.3%) using data to 3.3 Å. The CNS package (ref. 26) was used for refinement. A 2F_o-F_c map generated after rigid body refinement of the polyaniline model allowed placement of most sidechains. After a cycle of simulated annealing, the hypervariable loops were included. Density for the peptide was clear at this point and could be fitted unambiguously. Following another cycle of annealing, positional and B-factor refinement, waters were

12

included where peaks of >3.5 were found in a difference map at an appropriate distance from a donor or acceptor atom.

The structure of the uncomplexed Fab'2F5 was solved by molecular replacement using the refined Fab'2F5 complex minus peptide as the search model. Correlation coefficient was 53.7, R=39.0%. Refinement followed the same procedure as for the complex. Statistics of data collection, processing and structure refinement are given in Table 2 below. The coordinates of the crystal structures have been deposited on April 9, 1999 in the Brookhaven Protein Data Bank under Accession Nos. 2F5A for the uncomplexed structure and 2F5B for the Fab'2F5-epitope complex.

10 Example 3

This Example demonstrates the utility of the three-dimensional structural information of Kalinge's epitope (Examples 1 and 2) in the rational design of constraint peptide-based vaccines.

15 1. ECDKWCS CLP-634 (SEQ ID No: 3)

Based on the structural information, the Kalinge's epitope may be locked with a disulfide bridge between positions 2 and 6 in the peptide ECDKWCS (CLP-634).

The linear peptide ECDKWCS was synthesised manually, on PAM support, by using a standard Solid Phase Peptide Synthesis methodology, with a t-Boc strategy. The crude peptide was cleaved off the resin by high-HF procedure. The crude material (54 mg) was dissolved in methanol (500 mL), 50 mM iodine in methanol was added dropwise, with stirring, until solution became pale-yellow. After 1 min of stirring, Dowex IX2-200 (acetate) resin (approx. 9 g) was added. The stirring was continued until solution became colourless. The resin was filtered off, 50 mL of water was added, the mixture was concentrated *in vacuo*, frozen and lyophilised. The crude cyclic peptide was purified by RP-HPLC.

2. EdapDKWES CLP-1309 (SEQ ID No: 4)

Based on the structural information, the Kalinge's peptide also may be constrained with a lactam bond between positions 2 and 6 in the peptide EdapDKWES (CLP-1309).

13

The peptide: $\text{t-Boc-Glu(OBzl)-Dap(Fmoc)-Asp(OBzl)-Lys(2Cl-Cbz)-Trp(For)-Glu(Orn)-Ser(Bzl)-RESIN}$ was assembled on a PAM solid support. Sidechains of Dap(2) and Glu(6) were subsequently deprotected by treatment with 25% piperidine. The sidechain cyclization was performed on the resin by adding four equivalents of HBTU and 8 equivalents of DIEA and shaking the mixture overnight. The cyclic peptide was cleaved off the resin by a standard HF procedure and the crude product was purified by RP-HPLC.

Abbreviations used in this Example are:

Dap = diaminopropionic acid

10 HBTU = O-Benzotriazolyl-N,N,N',N'-tetramethyluronium

Hexafluorophosphate

DIEA = Di-isopropylethylamine

PAM = 4-Hydroxyethyl-phenylacetamidomethyl resin

Bzl = Benzyl

15 2-Cl-Cbz = 2-Chlorobenzoyloxy carbonyl

For = Fomyl

t-Boc = t-Butyloxycarbonyl

Fmoc = Fluorenylmethoxycarbonyl

Fm = Fluorenylmethyl

20 Both peptides CLP-634 and CLP-1309 were found to be capable of forming an immuno-complex with monoclonal antibody 2F5 and were subsequently co-crystallized with the Fab' fragment. These results indicated that the constrained peptides may mimic the Kalinga's epitope and would be useful as peptide-based vaccines.

25 Example 4

This Example demonstrates the formation of constrained peptide-carrier conjugates, for use as anti-HIV vaccines.

In order to conjugate the constrained peptide CLP-1309 (Example 3) to a carrier protein, a tetra-peptide Cys-Gly-Gly-Gly was linked to CLP-1309 at the N-terminal end and the resultant peptide was named as CLP-1491. Similarly, a tetra-peptide Gly-Gly-Gly-Cys was linked to CLP-1309 at the C-terminal end, and so the resultant peptide was named as CLP-1492.

14

Fifty microlitre of m-maleimidobenzoyl-N-hydroxysuccinimide (MBS, Pierce, 2 mg, 6.3 mmol in 1 mL of tetrahydrofuran or methanol) was added to a protein solution (approximately 10 mg of Hln47 or tetanus toxoid in 2 mL of 0.1 M phosphate buffer, pH 7.5). The reaction mixture was stirred for 30 min at room temperature under argon. The reaction mixture was applied to a Sephadex G-25 column (20 x 300 mm) equilibrated with 20 mM ammonium bicarbonate buffer, pH 7.2 and eluted with the same buffer. Elution was monitored by absorbance at 220 nm, and the eluted protein peak was pooled. The number of maleimide groups incorporated into the carrier was determined by adding excess 2-mercaptoethanol to the activated carrier-MBS and back-titrating the excess using a modified Ellman's method (ref. 31).

A general protocol for peptide-carrier conjugates has been described (ref. 32). Briefly, synthetic peptide (1 mg/mL) in degassed PBS buffer, pH 7.5 mixed with carrier-MBS (1 mg/mL). The reaction mixture was stirred overnight at room temperature under argon atmosphere. Excess N-ethyl-maleimide (Aldrich) was added to quench the reaction, and stirring continued for an additional hour. The insoluble precipitate was filtered off, and the filtrate was subjected to gel filtration chromatography using a Sephadex G-25 column. The peptide-carrier conjugate was collected. The molar ratio of carrier to peptide was determined by using amino acid analysis.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the crystal structure of the Fab2F5 fragment has been elucidated, both in uncomplexed form and complexed with the epitope ELDKWAS, and peptides synthesized to correspond to the constrained structure of the peptide-protein interactions. Modifications are possible within the scope of this invention.

15

Table 1

AQLUTSPSS LSASVDRIT ITCRASQVT SALAWYRKP GSPPOLLIYD
 ASLSBSVPS RPSGSGSSTE FTLITSTLAP EDPATYCCQ LHFYPTFGG
 GTRVDVRYT AASVFIIPP SDBLKSQTR SYVCLANIFY PREAYOMKY
 DNALQGNQD ESYTEDSD SDYLSSTLT LSKADYERHK VYACVTHOG
 LSPTVTSFN RDEC (SEQ ID No.: 6)
 RILKESGP LVKPTQTTLT TCSFGSPSIS DFGVGVNIR OPRKALENL
 ALIYSDDERK YSPSLNRLT ITDTSKNOV VLVNTRVSPV DPAIFYCAHR
 RGPITLFGVP IARGPVNAD VMQDITVTI SSASTKPSV FPLASRST
 SGTALAGLU VKQYPEPVT VSMNSGALTS GVHTFPAYIQ SGLYSLSBV
 VTPSSSLCT QTYICNVNKH PSNTKVDKXV EPKCDKTHI CPEDAPELL
 GGPVFLFPP KFKOTLWISR TPHTCCVND VSHEDDEPVT NAYVDGVEH
 NAKIKREEQ VNSTYRVSV LVTLHQDMLN GREYKCNYSN KAPPAIEKT
 ISKXGQPRE POVYTLPPSR DELTKQVSL TGLVKGFYPS DIAVBSNG
 OPEWYKTP PYLDSGSPF LYSKLTVDKS RMQDGNVFSK SVHBSALANH
 YTKSLSLSP GK (SEQ ID No.: 7)

16

Table 2
Data Collection, Processing and Structure Refinement Parameters

Compound	Fab'2F5	Fab'2F5-ELDKWAS
Crystal system; space group	orthorhombic; P2 ₁ 2 ₁ 2 ₁	orthorhombic; P2 ₁ 2 ₁ 2 ₁
Unit cell (Å)	a=63.3 b=76.3 c=94.4	a=58.0; b=65.0, c=175.6
Resolution range (Å)	20.0 - 2.05	12.0 - 2.0
# of reflections	89376	118126
# unique reflections	28045	41062
Completeness; completeness top bin (%)	92; 93	90; 92
R _{sym} ; R _{sym} top bin (%)	7.0; 31.3	3.5; 16.6
-cutoff	0.0	1.0
% data in test set	5	5
# water molecules in model	268	357
R, R _{free}	0.23, 0.27	0.22, 0.25
Rmsd bonds (Å); angles (°)	0.007; 1.4	0.010; 1.5

17

Table 3

ATOM	2399	N	ALA	H	98	-0.049	39.377	79.646	1.00	21.77
ATOM	2400	CA	ALA	H	98	1.135	39.444	80.483	1.00	21.70
ATOM	2401	CB	ALA	H	98	2.361	39.794	79.633	1.00	21.47
ATOM	2402	C	ALA	H	98	.979	40.460	81.598	1.00	21.53
ATOM	2403	O	ALA	H	98	.223	41.419	81.490	1.00	21.06
ATOM	2404	N	HIS	H	99	1.731	40.229	82.660	1.00	21.37
ATOM	2405	CA	HIS	H	99	1.719	41.072	83.861	1.00	21.17
ATOM	2406	CB	HIS	H	99	1.956	40.169	85.059	1.00	21.35
ATOM	2407	CG	HIS	H	99	2.229	40.897	86.336	1.00	21.04
ATOM	2408	CD	HIS	H	99	1.395	41.316	87.319	1.00	20.90
ATOM	2409	ND1	HIS	H	99	3.504	41.224	86.746	1.00	21.12
ATOM	2410	CE1	HIS	H	99	3.446	41.608	87.931	1.00	20.64
ATOM	2411	NE2	HIS	H	99	2.179	41.876	88.301	1.00	20.95
ATOM	2412	C	HIS	H	99	2.748	42.394	83.773	1.00	21.64
ATOM	2413	O	HIS	H	99	3.831	42.026	83.207	1.00	21.32
ATOM	2414	N	ARG	H	100	2.379	43.355	84.306	1.00	21.79
ATOM	2415	CA	ARG	H	100	3.292	44.483	84.354	1.00	22.26
ATOM	2416	CB	ARG	H	100	2.824	45.673	83.507	1.00	22.31
ATOM	2417	CG	ARG	H	100	3.884	46.772	83.478	1.00	22.62
ATOM	2418	CD	ARG	H	100	3.486	48.026	82.712	1.00	22.45
ATOM	2419	NE	ARG	H	100	4.626	48.941	82.623	1.00	22.59
ATOM	2420	C2	ARG	H	100	4.569	50.179	82.133	1.00	22.62
ATOM	2421	NH1	ARG	H	100	3.425	50.676	81.684	1.00	22.75
ATOM	2422	NH2	ARG	H	100	5.674	50.910	82.055	1.00	23.15
ATOM	2423	C	ARG	H	100	3.363	44.906	85.805	1.00	22.74
ATOM	2424	O	ARG	H	100	2.337	45.128	86.460	1.00	22.03
ATOM	2425	N	ARG	H	101	4.579	45.001	86.304	1.00	23.46
ATOM	2426	CA	ARG	H	101	4.809	45.388	87.678	1.00	24.42

18

ATOM	2427	CB	ARG	H	101	6.287	45.169	88.017	1.00	25.61
ATOM	2428	CG	ARG	H	101	6.557	44.099	89.047	1.00	27.15
ATOM	2429	CD	ARG	H	101	7.573	43.067	88.572	1.00	28.68
ATOM	2430	NE	ARG	H	101	8.851	43.615	88.118	1.00	29.23
ATOM	2431	CZ	ARG	H	101	9.867	42.858	87.697	1.00	29.78
ATOM	2432	NH1	ARG	H	101	9.747	41.535	87.681	1.00	30.18
ATOM	2433	NH2	ARG	H	101	11.001	43.410	87.276	1.00	29.91
ATOM	2434	C	ARG	H	101	4.448	46.846	87.902	1.00	24.54
ATOM	2435	O	ARG	H	101	4.544	47.668	86.996	1.00	23.94
ATOM	2436	N	GLY	H	102	4.014	47.156	89.118	1.00	25.02
ATOM	2437	CA	GLY	H	102	3.709	48.529	89.453	1.00	26.02
ATOM	2438	C	GLY	H	102	4.957	49.055	90.136	1.00	27.10
ATOM	2439	O	GLY	H	102	5.889	48.280	90.375	1.00	26.58
ATOM	2440	N	PRO	H	103	5.031	50.357	90.449	1.00	27.97
ATOM	2441	CD	PRO	H	103	4.057	51.435	90.215	1.00	28.46
ATOM	2442	CA	PRO	H	103	6.218	50.901	91.111	1.00	29.02
ATOM	2443	CB	PRO	H	103	5.863	52.379	91.269	1.00	28.75
ATOM	2444	CG	PRO	H	103	4.982	52.630	90.056	1.00	28.56
ATOM	2445	C	PRO	H	103	6.458	50.226	92.457	1.00	30.21
ATOM	2446	O	PRO	H	103	5.515	49.927	93.185	1.00	30.26
ATOM	2447	N	THR	H	104	7.723	49.967	92.772	1.00	31.28
ATOM	2448	CA	THR	H	104	8.073	49.360	94.048	1.00	32.89
ATOM	2449	CB	THR	H	104	9.586	49.042	94.115	1.00	32.77
ATOM	2450	OD1	THR	H	104	9.888	48.014	93.167	1.00	33.00
ATOM	2451	CD2	THR	H	104	9.987	48.579	95.514	1.00	32.60
ATOM	2452	C	THR	H	104	7.720	50.366	95.141	1.00	33.71
ATOM	2453	O	THR	H	104	7.978	51.559	94.994	1.00	33.67
ATOM	2454	N	THR	H	105	7.123	49.889	96.225	1.00	35.02
ATOM	2455	CA	THR	H	105	6.745	50.769	97.321	1.00	36.43

19

ATOM	2456	CB	THR	H	105	5.217	50.723	97.589	1.00	36.53
H										
ATOM	2457	OG1	THR	H	105	4.837	49.399	97.990	1.00	36.95
H										
ATOM	2458	CG2	THR	H	105	4.437	51.116	96.334	1.00	36.64
H										
ATOM	2459	C	THR	H	105	7.470	50.384	98.609	1.00	37.35
H										
ATOM	2460	O	THR	H	105	7.892	49.242	98.773	1.00	37.48
H										
ATOM	2461	N	LEU	H	106	7.625	51.354	99.506	1.00	38.42
H										
ATOM	2462	CA	LEU	H	106	8.264	51.133	100.804	1.00	39.62
H										
ATOM	2463	CB	LEU	H	106	9.633	51.813	100.877	1.00	39.53
H										
ATOM	2464	CG	LEU	H	106	10.385	51.596	102.199	1.00	39.63
H										
ATOM	2465	CD1	LEU	H	106	10.643	50.107	102.396	1.00	39.65
H										
ATOM	2466	CD2	LEU	H	106	11.694	52.362	102.193	1.00	39.35
H										
ATOM	2467	C	LEU	H	106	7.319	51.756	101.825	1.00	40.38
H										
ATOM	2468	O	LEU	H	106	7.113	52.973	101.828	1.00	40.43
H										
ATOM	2469	N	PHE	H	107	6.753	50.916	102.687	1.00	41.38
H										
ATOM	2470	CA	PHE	H	107	5.784	51.366	103.679	1.00	42.27
H										
ATOM	2471	CB	PHE	H	107	6.443	52.268	104.774	1.00	43.05
H										
ATOM	2472	CG	PHE	H	107	7.522	51.468	105.525	1.00	43.75
H										
ATOM	2473	CD1	PHE	H	107	8.855	51.624	105.155	1.00	44.10
H										
ATOM	2474	CD2	PHE	H	107	7.202	50.645	106.585	1.00	44.17
H										
ATOM	2475	CE1	PHE	H	107	9.857	50.935	105.829	1.00	44.32
H										
ATOM	2476	CE2	PHE	H	107	8.195	49.948	107.265	1.00	44.42
H										
ATOM	2477	CZ	PHE	H	107	9.527	50.094	106.887	1.00	44.38
H										
ATOM	2478	C	PHE	H	107	4.736	52.194	102.946	1.00	42.37
H										
ATOM	2479	O	PHE	H	107	4.355	53.276	103.390	1.00	42.68
H										
ATOM	2480	N	GLY	H	108	4.258	51.681	101.799	1.00	42.27
H										
ATOM	2481	CA	GLY	H	108	3.290	52.368	101.015	1.00	42.09
H										
ATOM	2482	C	GLY	H	108	3.777	53.434	100.051	1.00	41.71
H										
ATOM	2483	O	GLY	H	108	3.065	53.782	99.112	1.00	42.19
H										
ATOM	2484	N	VAL	H	109	4.979	53.957	100.260	1.00	40.92
H										

WO 00/61618

20

ATOM	2485	CA	VAL	H	109	5.491	54.996	99.373	1.00	40.10
ATOM	2486	CB	VAL	H	109	6.406	55.988	100.138	1.00	40.30
ATOM	2487	CGI	VAL	H	109	6.868	57.097	99.209	1.00	40.21
ATOM	2488	CG2	VAL	H	109	5.667	56.568	101.330	1.00	40.54
ATOM	2489	C	VAL	H	109	6.275	54.441	98.184	1.00	39.35
ATOM	2490	O	VAL	H	109	7.226	53.678	98.153	1.00	39.16
ATOM	2491	N	PRO	H	110	5.867	54.805	96.956	1.00	38.61
ATOM	2492	CD	PRO	H	110	4.728	55.654	96.569	1.00	38.51
ATOM	2493	CA	PRO	H	110	6.567	54.329	95.757	1.00	37.67
ATOM	2494	CB	PRO	H	110	5.728	54.922	94.629	1.00	37.96
ATOM	2495	CG	PRO	H	110	5.221	56.214	95.258	1.00	38.42
ATOM	2496	C	PRO	H	110	7.988	54.887	95.782	1.00	36.69
ATOM	2497	O	PRO	H	110	8.179	56.099	95.921	1.00	36.53
ATOM	2498	N	ILE	H	111	8.977	54.006	95.654	1.00	35.32
ATOM	2499	CA	ILE	H	111	10.377	54.419	95.692	1.00	34.04
ATOM	2500	CB	ILE	H	111	11.087	53.834	96.297	1.00	34.06
ATOM	2501	CG2	ILE	H	111	10.441	54.361	98.204	1.00	34.21
ATOM	2502	CG1	ILE	H	111	11.017	52.305	96.876	1.00	34.03
ATOM	2503	CD1	ILE	H	111	11.776	51.607	97.990	1.00	33.88
ATOM	2504	C	ILE	H	111	11.180	54.009	94.463	1.00	33.02
ATOM	2505	O	ILE	H	111	12.367	54.322	94.365	1.00	33.88
ATOM	2506	N	ALA	H	112	10.551	53.296	93.556	1.00	31.78
ATOM	2507	CA	ALA	H	112	11.255	52.862	92.338	1.00	30.94
ATOM	2508	CB	ALA	H	112	12.149	51.670	92.667	1.00	30.98
ATOM	2509	C	ALA	H	112	10.300	52.496	91.213	1.00	30.17
ATOM	2510	O	ALA	H	112	9.394	51.681	91.398	1.00	30.19
ATOM	2511	N	ARG	H	113	10.506	53.091	90.046	1.00	29.21
ATOM	2512	CA	ARG	H	113	9.651	52.797	88.905	1.00	28.40
ATOM	2513	CB	ARG	H	113	9.199	54.100	88.239	1.00	28.78

21

ATOM	2514	CG	ARG H 113	10.337	55.009	87.853	1.00	28.97
H								
ATOM	2515	CD	ARG H 113	9.850	56.258	87.132	1.00	29.05
H								
ATOM	2516	NR	ARG H 113	10.971	57.131	86.821	1.00	29.19
H								
ATOM	2517	CZ	ARG H 113	10.940	58.104	85.916	1.00	29.34
H								
ATOM	2518	NH1	ARG H 113	9.831	58.339	85.217	1.00	28.91
H								
ATOM	2519	NH2	ARG H 113	12.029	58.835	85.702	1.00	29.08
H								
ATOM	2520	C	ARG H 113	10.353	51.901	87.892	1.00	27.85
H								
ATOM	2521	O	ARG H 113	9.746	51.462	86.920	1.00	27.45
H								
ATOM	2522	N	GLY H 114	11.632	51.620	88.122	1.00	27.08
H								
ATOM	2523	CA	GLY H 114	12.367	50.768	87.203	1.00	26.56
H								
ATOM	2524	C	GLY H 114	11.655	49.456	86.897	1.00	26.06
H								
ATOM	2525	O	GLY H 114	11.588	49.036	85.738	1.00	25.97
H								
ATOM	2526	N	PRO H 115	11.132	48.763	87.918	1.00	25.66
H								
ATOM	2527	CD	PRO H 115	11.212	49.041	89.362	1.00	25.59
H								
ATOM	2528	CA	PRO H 115	10.432	47.497	87.700	1.00	25.02
H								
ATOM	2529	CB	PRO H 115	10.028	47.087	89.119	1.00	25.85
H								
ATOM	2530	CG	PRO H 115	9.921	48.435	89.838	1.00	26.45
H								
ATOM	2531	C	PRO H 115	9.239	47.534	86.734	1.00	24.10
H								
ATOM	2532	O	PRO H 115	8.808	46.495	86.252	1.00	23.75
H								
ATOM	2533	N	VAL H 116	8.700	48.710	86.446	1.00	22.92
H								
ATOM	2534	CA	VAL H 116	7.565	48.764	85.531	1.00	22.26
H								
ATOM	2535	CB	VAL H 116	6.730	50.062	85.719	1.00	21.84
H								
ATOM	2536	CG1	VAL H 116	6.401	50.266	87.199	1.00	21.48
H								
ATOM	2537	CG2	VAL H 116	7.472	51.255	85.150	1.00	20.99
H								
ATOM	2538	C	VAL H 116	8.022	48.696	84.066	1.00	22.08
H								
ATOM	2539	O	VAL H 116	7.198	48.513	83.166	1.00	22.38
H								
ATOM	2540	N	ASN H 117	9.327	48.824	83.826	1.00	21.63
H								
ATOM	2541	CA	ASN H 117	9.826	48.813	82.455	1.00	21.64
H								
ATOM	2542	CB	ASN H 117	11.071	49.697	82.338	1.00	21.90
H								

22

ATOM	2543	CG	ASN H 117	10.748	51.173	82.526	1.00	22.54
H								
ATOM	2544	OD1	ASN H 117	9.686	51.630	82.116	1.00	22.65
H								
ATOM	2545	ND2	ASN H 117	11.673	51.922	83.115	1.00	22.26
H								
ATOM	2546	C	ASN H 117	10.070	47.451	81.814	1.00	21.39
H								
ATOM	2547	O	ASN H 117	11.186	47.122	81.396	1.00	21.27
H								
ATOM	2548	N	ALA H 118	8.984	46.691	81.716	1.00	21.30
H								
ATOM	2549	CA	ALA H 118	8.964	45.364	81.123	1.00	21.19
H								
ATOM	2550	CB	ALA H 118	10.093	44.511	81.695	1.00	21.58
H								
ATOM	2551	C	ALA H 118	7.632	44.713	81.466	1.00	21.25
H								
ATOM	2552	O	ALA H 118	6.898	45.197	82.333	1.00	21.59
H								
ATOM	2553	N	MET H 119	7.329	43.630	80.759	1.00	21.14
H								
ATOM	2554	CA	MET H 119	6.153	42.814	81.012	1.00	21.00
H								
ATOM	2555	CB	MET H 119	5.413	42.486	79.712	1.00	21.35
H								
ATOM	2556	CG	MET H 119	4.782	43.691	79.004	1.00	21.59
H								
ATOM	2557	SD	MET H 119	3.738	44.767	80.053	1.00	22.00
H								
ATOM	2558	CE	MET H 119	4.880	45.836	80.681	1.00	24.35
H								
ATOM	2559	C	MET H 119	6.907	41.594	81.542	1.00	21.33
H								
ATOM	2560	O	MET H 119	7.499	40.829	80.773	1.00	21.24
H								
ATOM	2561	N	ASP H 120	6.894	41.430	82.858	1.00	21.43
H								
ATOM	2562	CA	ASP H 120	7.679	40.381	83.500	1.00	21.62
H								
ATOM	2563	CB	ASP H 120	8.014	40.819	84.932	1.00	21.73
H								
ATOM	2564	CG	ASP H 120	6.806	40.826	85.840	1.00	22.35
H								
ATOM	2565	OD1	ASP H 120	5.661	40.878	85.330	1.00	21.92
H								
ATOM	2566	OD2	ASP H 120	7.011	40.807	87.075	1.00	21.94
H								
ATOM	2567	C	ASP H 120	7.209	38.931	83.499	1.00	21.67
H								
ATOM	2568	O	ASP H 120	8.020	38.027	83.668	1.00	21.12
H								

23

Table 4

EUDKMAS:									
ATOM	3373	CB	GLU	P	1	.169	60.111	75.304	1.00 29.50 P
ATOM	3374	CG	GLU	P	1	-.450	58.935	76.069	1.00 30.79 P
ATOM	3375	CD	GLU	P	1	-1.151	57.917	75.185	1.00 31.68 P
ATOM	3376	OE1	GLU	P	1	-.571	57.477	74.172	1.00 32.86 P
ATOM	3377	OE2	GLU	P	1	2.288	57.530	75.519	1.00 31.76 P
ATOM	3378	C	GLU	P	1	2.442	59.065	75.475	1.00 27.76 P
ATOM	3379	O	GLU	P	1	2.777	57.902	75.230	1.00 27.40 P
ATOM	3380	N	GLU	P	1	1.201	58.964	73.347	1.00 28.40 P
ATOM	3381	CA	GLU	P	1	1.473	59.802	74.549	1.00 28.51 P
ATOM	3382	N	LEU	P	2	2.882	59.739	76.537	1.00 27.14 P
ATOM	3383	CA	LEU	P	2	3.825	59.156	77.497	1.00 26.40 P
ATOM	3384	CB	LEU	P	2	4.343	60.235	78.462	1.00 26.88 P
ATOM	3385	CG	LEU	P	2	5.264	61.329	77.913	1.00 27.33 P
ATOM	3386	CD1	LEU	P	2	5.473	62.406	78.981	1.00 27.63 P
ATOM	3387	CD2	LEU	P	2	6.590	60.720	77.491	1.00 27.68 P
ATOM	3388	C	LEU	P	2	3.239	58.008	78.317	1.00 25.81 P
ATOM	3389	O	LEU	P	2	2.049	58.000	78.625	1.00 25.51 P
ATOM	3390	N	ASP	P	3	4.089	57.047	78.676	1.00 24.98 P
ATOM	3391	CA	ASP	P	3	3.676	55.898	79.480	1.00 24.32 P
ATOM	3392	CB	ASP	P	3	4.873	54.973	79.733	1.00 23.70 P
ATOM	3393	CG	ASP	P	3	4.531	53.803	80.642	1.00 23.27 P
ATOM	3394	OD1	ASP	P	3	3.595	53.040	80.302	1.00 22.76 P
ATOM	3395	OD2	ASP	P	3	5.191	53.643	81.693	1.00 21.86 P
ATOM	3396	C	ASP	P	3	3.109	56.356	80.824	1.00 24.44 P
ATOM	3397	O	ASP	P	3	3.351	57.484	81.263	1.00 24.24 P
ATOM	3398	N	LYS	P	4	2.380	55.466	81.489	1.00 24.58 P
ATOM	3399	CA	LYS	P	4	1.784	55.778	82.784	1.00 25.00 P
ATOM	3400	CB	LYS	P	4	1.079	54.543	83.350	1.00 24.68 P
ATOM	3401	CG	LYS	P	4	.247	54.779	84.613	1.00 24.80 P
ATOM	3402	CD	LYS	P	4	-.464	53.485	85.037	1.00 24.50 P
ATOM	3403	CE	LYS	P	4	-1.508	53.723	86.133	1.00 24.83 P
ATOM	3404	NZ	LYS	P	4	-2.572	54.671	85.678	1.00 24.26 P
ATOM	3405	C	LYS	P	4	2.816	56.253	83.806	1.00 25.53 P
ATOM	3406	O	LYS	P	4	2.528	57.124	84.622	1.00 25.08 P
ATOM	3407	N	TRP	P	5	4.020	55.693	83.753	1.00 25.97 P
ATOM	3408	CA	TRP	P	5	5.030	56.046	84.743	1.00 27.09 P
ATOM	3409	CB	TRP	P	5	5.639	54.756	85.307	1.00 26.62 P

24

ATOM	3410	CG	TRP	P	5	4.580	53.754	85.684	1.00 26.36 P
ATOM	3411	CD2	TRP	P	5	3.646	53.863	86.766	1.00 26.15 P
ATOM	3412	CE2	TRP	P	5	2.774	52.752	86.682	1.00 25.96 P
ATOM	3413	CE3	TRP	P	5	3.461	54.795	87.798	1.00 26.24 P
ATOM	3414	CD1	TRP	P	5	4.247	52.607	85.006	1.00 26.28 P
ATOM	3415	NE1	TRP	P	5	3.164	52.003	85.602	1.00 25.88 P
ATOM	3416	CE2	TRP	P	5	1.728	52.545	88.706	1.00 26.20 P
ATOM	3417	CE3	TRP	P	5	2.415	54.593	88.597	1.00 25.91 P
ATOM	3418	CH2	TRP	P	5	1.564	53.477	88.597	1.00 25.91 P
ATOM	3419	C	TRP	P	5	6.137	56.995	84.280	1.00 27.96 P
ATOM	3420	O	TRP	P	5	7.123	57.182	84.985	1.00 27.77 P
ATOM	3421	N	ALA	P	6	5.967	57.598	83.107	1.00 28.24 P
ATOM	3422	CA	ALA	P	6	6.957	58.534	82.571	1.00 30.79 P
ATOM	3423	CB	ALA	P	6	6.738	58.733	81.077	1.00 30.55 P
ATOM	3424	C	ALA	P	6	6.919	59.890	83.277	1.00 31.11 P
ATOM	3425	O	ALA	P	6	5.904	60.273	83.848	1.00 31.54 P
ATOM	3426	N	SER	P	7	8.040	60.601	83.213	1.00 31.55 P
ATOM	3427	CA	SER	P	7	8.206	61.923	83.812	1.00 35.02 P
ATOM	3428	CB	SER	P	7	7.007	62.821	83.481	1.00 35.56 P
ATOM	3429	CG	SER	P	7	6.922	63.058	82.085	1.00 36.31 P
ATOM	3430	C	SER	P	7	8.388	61.868	85.317	1.00 35.70 P
ATOM	3431	O	SER	P	7	9.555	61.945	85.772	1.00 35.92 P
ATOM	3432	OT	SER	P	7	7.357	61.724	86.013	1.00 36.58 P

25

Table 5

EUDRWAS:

ATOM 3265	CB	GLU	P	1	.001	59.852	75.796	1.00	71.00	P
ATOM 3266	CG	GLU	P	1	-.479	58.562	76.462	1.00	71.58	P
ATOM 3267	CD	GLU	P	1	-1.144	57.609	75.484	1.00	71.95	P
ATOM 3268	OEL	GLU	P	1	-.554	57.311	74.431	1.00	72.48	P
ATOM 3269	OEL	GLU	P	1	-2.260	57.134	75.803	1.00	71.87	P
ATOM 3270	C	GLU	P	1	2.326	58.990	75.760	1.00	36.82	P
ATOM 3271	O	GLU	P	1	2.717	57.867	75.436	1.00	36.76	P
ATOM 3272	N	GLU	P	1	.985	59.009	73.662	1.00	37.23	P
ATOM 3273	CA	GLU	P	1	1.270	59.720	74.941	1.00	37.14	P
ATOM 3274	N	LEU	P	2	2.775	59.627	76.833	1.00	33.88	P
ATOM 3275	CA	LEU	P	2	3.783	59.034	77.702	1.00	33.45	P
ATOM 3276	CB	LEU	P	2	4.389	60.114	78.611	1.00	61.37	P
ATOM 3277	CG	LEU	P	2	5.316	61.181	78.000	1.00	61.47	P
ATOM 3278	CD1	LEU	P	2	5.506	62.346	78.978	1.00	61.51	P
ATOM 3279	CD2	LEU	P	2	6.659	60.540	77.642	1.00	61.59	P
ATOM 3280	C	LEU	P	2	3.249	57.876	78.568	1.00	33.17	P
ATOM 3281	O	LEU	P	2	2.140	57.937	79.109	1.00	32.99	P
ATOM 3282	N	ASP	P	3	4.054	56.821	78.684	1.00	36.78	P
ATOM 3283	CA	ASP	P	3	3.700	55.666	79.496	1.00	36.51	P
ATOM 3284	CB	ASP	P	3	4.892	54.727	79.664	1.00	27.42	P
ATOM 3285	CG	ASP	P	3	4.583	53.569	80.597	1.00	27.10	P
ATOM 3286	OD1	ASP	P	3	3.676	52.778	80.258	1.00	26.93	P
ATOM 3287	OD2	ASP	P	3	5.235	53.460	81.668	1.00	26.53	P
ATOM 3288	C	ASP	P	3	3.285	56.155	80.868	1.00	36.57	P
ATOM 3289	O	ASP	P	3	3.595	57.280	81.245	1.00	36.49	P
ATOM 3290	N	ARG	P	4	2.628	55.288	81.629	1.00	47.13	P
ATOM 3291	CA	ARG	P	4	2.150	55.639	82.957	1.00	47.37	P
ATOM 3292	CB	ARG	P	4	1.309	54.495	83.516	1.00	57.30	P
ATOM 3293	CG	ARG	P	4	.545	54.865	84.764	1.00	57.28	P
ATOM 3294	CD	ARG	P	4	-.201	53.678	85.351	1.00	57.26	P
ATOM 3295	NE	ARG	P	4	-1.066	54.115	86.436	1.00	50.30	P
ATOM 3296	CZ	ARG	P	4	-1.736	53.309	87.256	1.00	50.30	P
ATOM 3297	NH1	ARG	P	4	-1.646	51.994	87.118	1.00	50.30	P
ATOM 3298	NH2	ARG	P	4	-2.495	53.822	88.227	1.00	50.30	P
ATOM 3299	C	ARG	P	4	3.238	56.014	83.971	1.00	47.65	P
ATOM 3300	O	ARG	P	4	3.016	56.861	84.840	1.00	47.39	P
ATOM 3301	N	TRP	P	5	4.412	55.402	83.873	1.00	41.46	P

26

ATOM 3302	CA	TRP	P	5	5.460	55.724	84.829	1.00	41.97	P
ATOM 3303	CB	TRP	P	5	6.039	54.431	85.387	1.00	45.39	P
ATOM 3304	CG	TRP	P	5	4.981	53.415	85.744	1.00	45.32	P
ATOM 3305	CD2	TRP	P	5	4.092	53.454	86.870	1.00	45.24	P
ATOM 3306	CE2	TRP	P	5	3.257	52.319	86.781	1.00	45.24	P
ATOM 3307	CE3	TRP	P	5	3.920	54.340	87.948	1.00	45.31	P
ATOM 3308	CD1	TRP	P	5	4.655	52.292	85.041	1.00	45.27	P
ATOM 3309	NE1	TRP	P	5	3.623	51.627	85.657	1.00	45.13	P
ATOM 3310	CZ2	TRP	P	5	2.266	52.044	87.724	1.00	45.22	P
ATOM 3311	CE2	TRP	P	5	2.931	54.064	88.891	1.00	45.30	P
ATOM 3312	CH2	TRP	P	5	2.117	52.924	88.769	1.00	45.34	P
ATOM 3313	C	TRP	P	5	6.582	56.618	84.264	1.00	42.36	P
ATOM 3314	O	TRP	P	5	7.669	56.695	84.834	1.00	42.32	P
ATOM 3315	N	ALA	P	6	6.296	57.305	83.157	1.00	47.84	P
ATOM 3316	CA	ALA	P	6	7.267	58.192	82.512	1.00	48.51	P
ATOM 3317	CB	ALA	P	6	6.977	58.286	81.026	1.00	39.87	P
ATOM 3318	C	ALA	P	6	7.290	59.597	83.117	1.00	49.00	P
ATOM 3319	O	ALA	P	6	6.372	60.000	83.838	1.00	49.16	P
ATOM 3320	N	SER	P	7	8.349	60.336	82.795	1.00	52.63	P
ATOM 3321	CA	SER	P	7	8.551	61.700	83.282	1.00	53.25	P
ATOM 3322	CB	SER	P	7	7.283	62.531	83.064	1.00	91.37	P
ATOM 3323	CG	SER	P	7	7.464	63.854	83.541	1.00	91.74	P
ATOM 3324	C	SER	P	7	8.937	61.727	84.765	1.00	53.52	P
ATOM 3325	O	SER	P	7	10.153	61.808	85.062	1.00	53.79	P
ATOM 3326	OT	SER	P	7	8.026	61.637	85.617	1.00	92.11	P

REFERENCES

1. Muster, T., et al., A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1, *J. Virol.*, 67, 6642-6647 (1993).
2. Muster, T., et al., Cross-neutralizing activity against divergent human immunodeficiency virus type 1 isolates induced by the gp41 sequence ELDKWAAS, *J. virology*, 68, 4031-4034 (1994).
3. Purtscher, M., et al., A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1 (HIV-1) AIDS Res. And Human Retroviruses, 10, 1651-1658 (1994).
4. Conley, A.J., et al., Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, an anti-gp41 human monoclonal antibody, *Proc. Natl. Acad. Sci. USA*, 91, 3348-3352 (1994).
5. Trkola, A., et al., Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IGG, *J. Virology*, 69, 6609-6617 (1995).
6. Burton D.R., A vaccine for HIV type 1: The antibody perspective, *Proc. Natl. Acad. Sci. USA*, 94, 10018-10023 (1997).
7. Mascola, J.R., et al. Potent and synergistic Neutralization of human immunodeficiency virus (HIV) type 1 primary isolates by hyperimmune anti-HIV immunoglobulin combined with monoclonal antibodies 2F5 and 2G12, *J. Virology*, 71, 7198-7206 (1997).
8. Eckhart, L., et al., Immunogenic presentation of a conserved gp41 epitope of human immunodeficiency virus type 1 on recombinant surface antigens of hepatitis B. virus, *J. of General Virology*, 77, 2001-2008 (1996).
9. Kunert, R., et al., Molecular characterization of five neutralizing anti-HIV type 1 antibodies: identification of nonconventional D segments in the human monoclonal antibodies 2G12 and 2F5, *AIDS Res. and Human Retroviruses*, 14, 1115-1128, (1998).
10. Richardson, J.S., The anatomy and taxonomy of protein structure, *Adv. Protein Chem.*, 34, 167-339, (1981).
11. HIV Sequence Database, Los Alamos National Laboratory, Theoretical Biology and Biophysics Group T-10, Los Alamos, New Mexico.
12. Nicholls, A., Honig, B., "GRASP", Columbia University.
13. Gallaher, W.R., et al., A general model for the transmembrane proteins of HIV and other retroviruses, *AIDS Res. And Human Retroviruses*, 5, 431-440 (1989).
14. Weissenhorn, W., et al., Atomic structure of the ectodomain from HIV-1 gp41, *Nature*, 387, 426-430 (1997).
15. Tan, K., et al., Atomic structure of a thermostable subdomain of HIV-1 gp41, *Proc. Natl. Acad. Sci. USA*, 94, 12303-12308 (1997).
16. Chan, d., et al., Core structure of gp41 from the HIV envelope glycoprotein, *Cell*, 89, 263-273 (1997).
17. Malaskevic, V.N., et al., Crystal structure of the simian immunodeficiency virus (SIV) gp41 core: Conserved helical interactions underlie the broad inhibitory activity of gp41 peptides, *Proc. Natl. Acad. Sci. USA*, 95, 9134-9139 (1998).
18. Yang, Z.N., et al., High resolution structure of simian immunodeficiency virus gp41 ectodomain, Abstracts, American Crystallographic Association Annual Meeting, 1998.
19. Caffrey, M., et al., Three-dimensional solution structure of the 44 kDa ectodomain of SIV gp41, the EMBO J., 17, 4572-4584 (1998).
20. Lim L., et al., The three-dimensional structure of glutathione-S-transferase of *Schistosoma japonicum* fused with a conserved neutralizing epitope of human immunodeficiency virus type 1, *Protein Science*, 3, 2233-2244 (1994).
21. Ernst W., et al., Baculovirus surface display: Construction and screening of a eukaryotic epitope library, *Nucl. Acids Res.* 26, 1718-1723 (1998).
22. Cook, J., et al., Recombinant antibodies with conformationally constrained HIV type 1 epitope inserts elicit glycoprotein 160-specific antibody responses *in vivo*, *AIDS Res. Human Retroviruses*, 13, 449-460 (1997).
23. Chan, D.E. & Kim, P.S., HIV entry and its inhibition, *Cell*, 93, 681-684 (1998).
24. Navaza, J., AMoRe- an automated package for molecular replacement, *Acta Crystallogr.*, A50, 157-163 (1994).
25. Jeffrey, P.D., et al., The X-ray structure of anti-tumour antibody in complex with antigen, *Nature Struct. Biol.*, 2, 466-471 (1995).

26. Brunger, A.T., et al., Crystallography and NMR system: A new software system for macromolecular structure determination, *Acta Cryst. D*, 54, 905-921 (1998).
27. Kraulis, P.J., MOLSCRIPT: a program to produce both detailed and schematic plots of protein structure, *J. Appl. Cryst.*, 24, 946-950 (1991).
28. Merritt, E.A. & Murphy, M.E.P. Raster 3D Version 2.0, A program for photorealistic Molecular graphics, *Acta Cryst. D*50, 869-873, (1994).
29. Jones, T.A. et al., *Acta Cryst. D*47, 110-119 (1991).
30. Evans, S.V., SETOR: hardware-lighted three-dimensional solid model representations of macromolecules, *J. Mol. Graph.*, 11, 134-8, (1993).
31. Riddles et al., (1983), *Methods Enzym.* 91:49-60.
32. Chong et al., (1991), *Mol. Immunol.* 28: 239-245.

CLAIMS

What we claim is:

1. An isolated crystal of the Fab' fragment of monoclonal antibody 2F5.
2. The isolated crystal of claim 1 consisting of molecules having the three-dimensional structure represented by Figure 1.
3. The isolated crystal of claim 1 consisting of molecules having the parameters defined in Table 2.
4. The isolated crystal of claim 3 consisting of molecules having a space group P2₁2₁2₁, with said cell dimensions a = 63.6 Å, b = 76.4 Å and c = 93.4 Å.
5. The isolated crystal of claim 1 consisting of molecules having a third hypervariable (V3) loop of the heavy chain comprising amino acid residues H98 to H120, as seen in Table 1, having a three-dimensional structure as shown in Figure 4.
6. The isolated crystal of claim 5 consisting of molecules wherein said V3 loop has the atomic coordinates shown in Table 3.
7. The isolated crystal of claim 1 consisting of molecules having the atomic coordinates deposited with the Protein Data Bank under Accession number 2F5A.
8. An isolated crystal of the Fab' fragment of monoclonal antibody 2F5 complexed with a peptide having the amino acid sequence ELDKWAS (SEQ ID No.: 1) or a functional analog thereof.
9. The isolated crystal of claim 8 consisting of molecules having a structure at the binding site of the peptide to the Fab' fragment as shown in Figure 3.
10. The isolated crystal of claim 8 consisting of molecules having the parameters defined in Table 2.
11. The isolated crystal of claim 10 consisting of molecules having a space group P2₁2₁2₁, with unit cell dimensions a = 58.0 Å, b = 65.0 Å and c = 175.6 Å.
12. The isolated crystal of claim 8 wherein said functional analog of said amino acid sequence ELDKWAS is selected from the group consisting of one in which lysine is replaced by arginine and one in which tryptophan is replaced by an amino acid selected from the group consisting of tyrosine, phenylalanine and uncharged histidine.
13. The isolated crystal of claim 8 wherein said peptide is ELDKWAS.

31

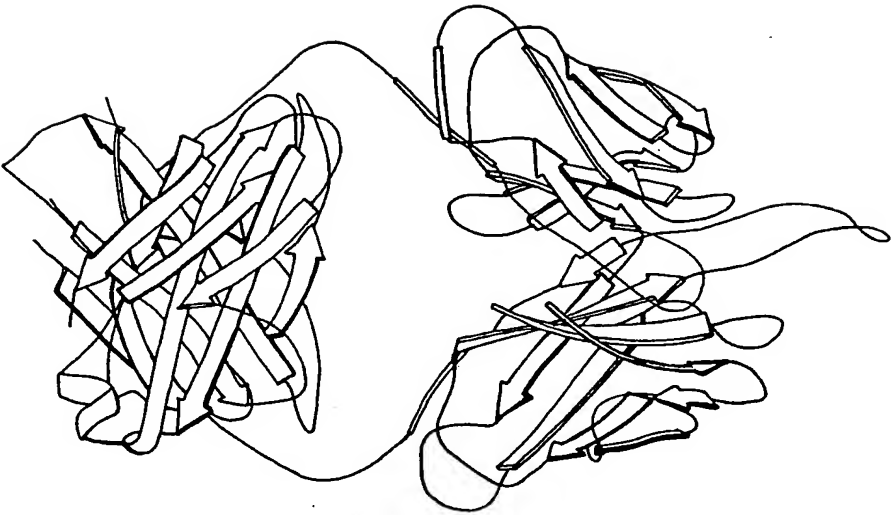
14. The isolated crystal of claim 13 wherein the Fab2F5 : ELDKWAS complex has the atomic coordinates of Table 4.
15. The isolated crystal of claim 8 wherein said peptide is ELDRWAS (SEQ ID No: 2).
16. The isolated crystal of claim 15 wherein said Fab2F5 : ELDRWAS complex has the atomic coordinates of Table 5.
17. The isolated crystal of claim 8 consisting of molecules having the atomic coordinates deposited with the Protein Data Bank under Accession number 2F5B.
18. A synthetic peptide which binds to monoclonal antibody 2F5 and which is constrained to provide a three-dimensional structure corresponding to that for the peptide ELDKWAS (SEQ ID No.: 1) shown in Figure 3.
19. The synthetic peptide of claim 18 which contains the amino acid sequence DKW or a functional analog thereof constrained in the slightly distorted β -turn configuration of said three-dimensional structure with the tryptophan and lysine sidechains stacked and parallel.
20. The synthetic peptide of claim 19 wherein at least one of said tryptophan and lysine amino acids is substituted by an amino acid which retains the peptide-protein interactions shown in Figure 3.
21. The synthetic peptide of claim 20 wherein said lysine residues is replaced by arginine.
22. The synthetic peptide of claim 20 wherein said tryptophan is replaced by tyrosine, phenylalanine or uncharged histidine.
23. The synthetic peptide of claim 20 wherein said lysine residue is replaced by arginine and said tryptophan is replaced by tyrosine, phenylalanine or uncharged histidine.
24. The synthetic peptide of claim 19 wherein said peptide contains a disulphide bridge to maintain said amino acid sequence DKW or functional analog thereof in the respective orientation of the amino acid residues as shown in Figure 3.
25. The synthetic peptide of claim 24 wherein said peptide has the amino acid sequence ECDKWCS (SEQ ID No.: 3) and said disulphide bridge is established between said cysteine (C) residues.

32

26. The synthetic peptide of claim 19 wherein said peptide contains a lactam bond to maintain said amino acid sequence DKW or functional analog thereof in the respective orientation of the amino acid residues as shown in Figure 3.
27. The synthetic peptide of claim 26 wherein said peptide has the formula EDapDKWES (SEQ ID No.: 5) and said lactam bond is formed between the Dap and glutamate (E) residues.
28. The synthetic peptide of claim 18 which is linked to a carrier protein.
29. A method of making a peptide binding to monoclonal antibody 2F5, which comprises:
 - co-crystallizing a Fab' fragment of the monoclonal antibody 2F5 with a peptide having the amino acid sequence ELDKWAS (SEQ ID No.: 1) or functional analog thereof to form a crystalline complex,
 - analyzing the crystalline complex to determine the three-dimensional orientation of the bound peptide in relation to the Fab' fragment, and
 - synthesizing a peptide containing at least amino acids DKW or functional analogs thereof constrained in the determined three-dimensional orientation.
30. The method of claim 29 wherein said functional analog of the peptide having the amino acid sequence ELDKWAS has the amino acid sequence ELDRWAS (SEQ ID No.: 2).

1/L

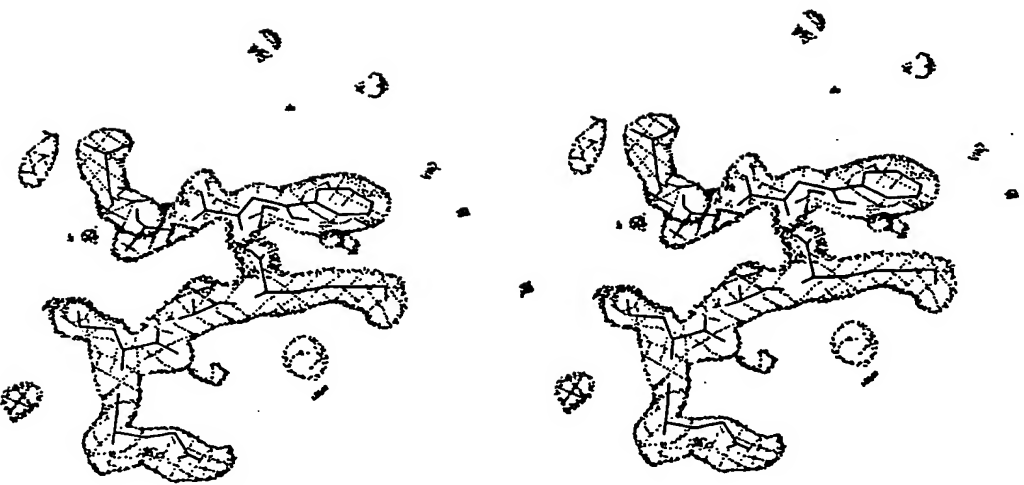
FIG.1



SUBSTITUTE SHEET (RULE 26)

2/L

FIG.2



SUBSTITUTE SHEET (RULE 26)

3/4

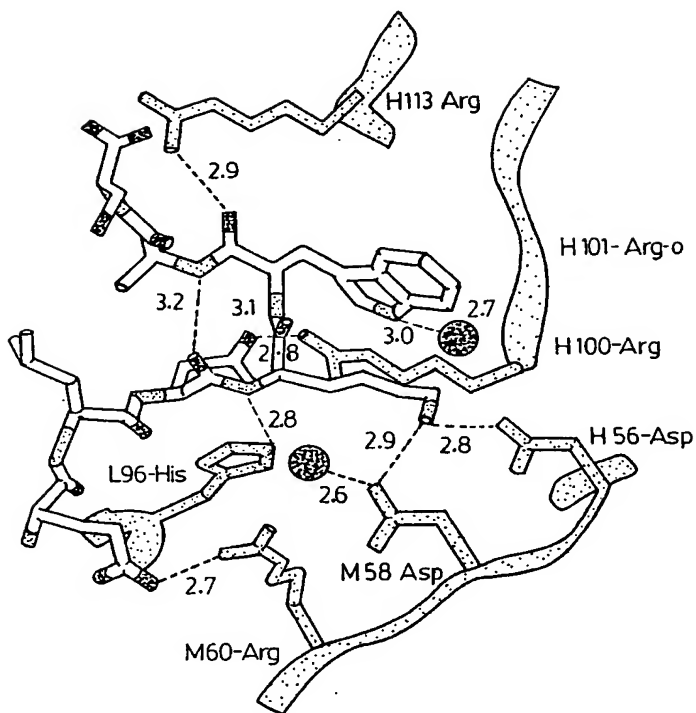


FIG.3

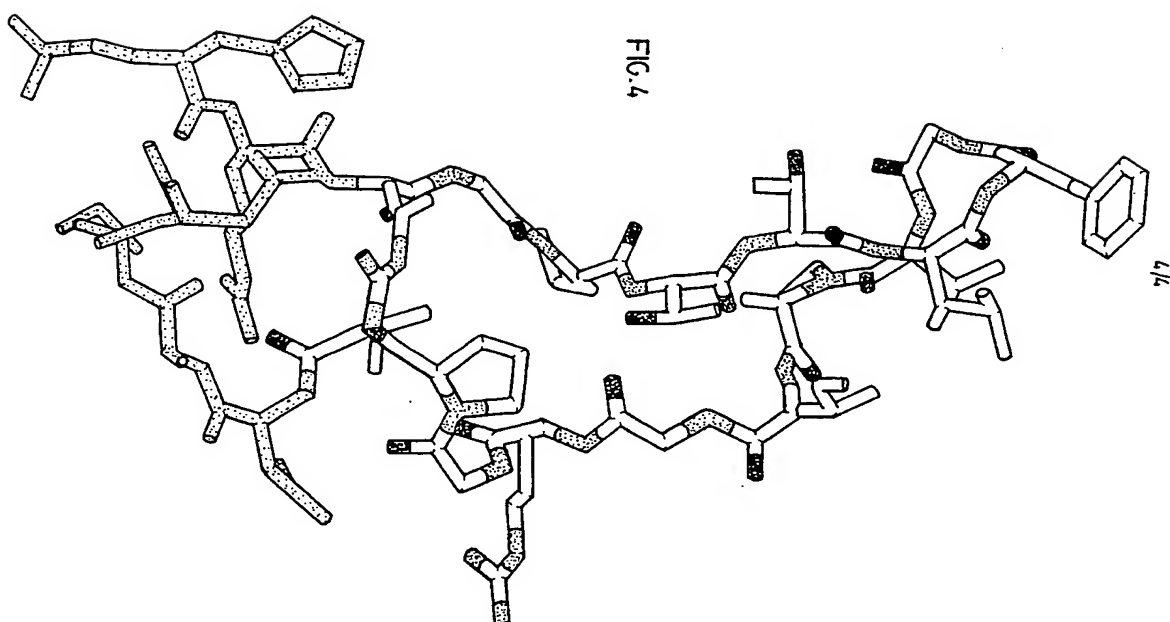


FIG.4

INTERNATIONAL SEARCH REPORT

 In-
tional Application No
PCT/CA 00/003358

 A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07K14/16 C07K16/10 C07K1/00

According to International Patent Classification (IPC) or to both national classification and IPC

 B. FIELD(S) SEARCHED
 Minimum documentation searched (classification system followed by classification symbols)
 IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data bases consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NO 96 02273 A (SCRIPPS RESEARCH INST) 1 February 1996 (1996-02-01) abstract; figure 6 page 102, line 10 - line 17	1-7
Y	MUSTER T. ET AL: "Cross-Neutralizing Activity against Divergent Human Immunodeficiency Virus Type 1 Isolates Induced by the gp41 Sequence ELKMAS." J. VIROL., vol. 68, no. 6, 1994, pages 4031-4034, XP000654602 abstract page 4031, column 1, paragraph 1 page 4033, column 2, paragraphs 2,3 -/---	1-17,25, 29,30

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

Special categories of cited documents:

- * " document defining the general state of the art which is not
 considered to be of particular relevance
 " document published on or after the international
 filing date
 " document which may grow directly or indirectly
 into an invention of the applicant or another
 person who is entitled to the priority of the
 " document relating to an oral disclosure, use, exhibition or
 other means
 " document published prior to the international filing date but
 later than the priority date claimed
 " document member of the same patent family
 " Date of mailing of the international search report

10 July 2000

24/07/2000

 Name and mailing address of the ISA
 European Patent Office, P.B. 5018 Patentamt 2
 NL - 2220 HH Rijswijk
 T: 31 (0)20 4852040 T: 31 651 4900 tel.
 Fax: (31) 20 340-3018

Authorized officer

Montreuil, M

From PCT/ISA210 (second revised July 1992)

INTERNATIONAL SEARCH REPORT

 In-
tional Application No
PCT/CA 00/003358

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	PURTSCHER M. ET AL: "Restricted antigenic variability of the epitope recognized by the neutralizing gp41 antibody 2F5." AIDS, vol. 10, 1996, pages 587-593, XP000916358 abstract page 589, column 1, paragraph 3 page 590, column 2, paragraph 4 -page 591, column 2, paragraph 1; figure 1 page 592, column 1, paragraph 2 - paragraph 3	1-17,25, 29,30
Y	CONLEY A.J. ET AL: "Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by 1A6-41-2F5, an anti-gp41 human monoclonal antibody." PNAS, vol. 91, 1994, pages 3348-3352, XP000652271 abstract page 3351, column 1, paragraph 3 -column 2, paragraph 1; figure 3	1-17,25, 29,30
Y	JEFFREY P.D. ET AL: "The X-ray structure of an anti-tumour antibody in complex with antigen" NATURE STRUCTURAL BIOLOGY, vol. 2, 1995, pages 466-471, XP000915894 cited in the application abstract page 466, column 2, paragraph 2	7-17
Y	US 5 831 034 A (STEINOL FRANZ ET AL) 3 November 1998 (1998-11-03) abstract column 1, line 18 - line 24 column 1, line 65 claims 1-4	1-7
Y	WO 95 07354 A (MUSTER THOMAS ;KATINGER HERMANN (AT): POLYMER SCIENT IMMUNO BIO FOS) 16 March 1995 (1995-03-16) abstract page 3, line 25 - line 33 page 4, line 10 - line 20 page 11, line 7 - line 22 -/---	1-17,25, 29,30

From PCT/ISA210 (continuation of second revised July 1992)

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/CA 00/00358C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT
Category: Citation of document, with indication, where appropriate, of the relevant passages
Relevant to claim No.

A	COOK J. ET AL.: "Recombinant Antibodies with Conformational Constrained HIV Type I Epitope Inserts Elicit Glycoprotein 160-Specific Antibody Responses in Vivo" AIDS RESEARCH AND HUMAN RETROVIRUSES, vol. 13, 1997, pages 449-460, XP000916356 abstract page 450, column 1, paragraph 2 page 452, column 2, paragraph 1; figure 1 page 456, column 2, paragraph 2 page 457, column 1, paragraph 3	
---	--	--

Form PCT/ISA42a (Continuation of second sheet) (July 1992)

page 3 of 3

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.
PCT/CA 00/00358

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9602273 A	01-02-1996	US 5652138 A	29-07-1997
		AU 706601 B	17-06-1999
		AU 2970095 A	16-02-1996
		AU 4875699 A	17-02-2000
		CA 2195454 A	01-02-1996
		EP 0777497 A	11-06-1997
		FI 970198 A	17-03-1997
		JP 10505229 T	26-05-1998
		NO 970221 A	18-03-1997
		US 5804440 A	08-09-1998
US 5831034 A	03-11-1998	AT 135743 T	15-04-1996
		DE 3855134 D	25-04-1996
		DE 3855134 T	02-10-1996
		WO 8904370 A	18-05-1989
		EP 0355140 A	28-02-1990
		JP 2502251 T	26-07-1990
		US 5735303 A	19-05-1998
WO 9507354 A	16-03-1995	AU 682893 B	23-10-1997
		AU 7696594 A	27-03-1995
		BR 9407531 A	26-08-1997
		CA 2171544 A	16-03-1995
		CN 1135237 A	06-11-1996
		CZ 9600741 A	17-07-1996
		EP 0729825 A	14-08-1996
		HU 76102 A	30-06-1997
		JP 9502348 T	11-03-1997

Form PCT/ISA42a (Patent family annex) (July 1992)

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant:

Defects in the images include but are not limited to the items checked:

- ☒ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☒ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.